Dilutions of the fusion protein test material (50 µl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 µg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 µC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the fusion protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

Example 43: Assays for Protease Activity

The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacryamide/0.1% SDS gels containing 1% gelain orcasein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis apear as clear areas agains the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mMNaPO$_4$,1mM EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

Example 44: Identifying Serine Protease Substrate Specificity

Methods known in the art or described herein may be used to determine the substrate

specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

Example 45: Ligand Binding Assays

The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

Example 46: Functional Assay in Xenopus Oocytes

Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/mi. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocytc) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca2+ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 47: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus

capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

Example 48: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 49: ATP-binding assay

The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (^{32}P-ATP) (5 mCi/μmol, ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenly-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

Example 50: Phosphorylation Assay

In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein

incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P-ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion portein of the invention is incubated with the protein substrate, ^{32}P-ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P-ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

Example 51: Detection of Phosphorylation Activity (Activation) of an Albumin Fusion Protein of the Invention in the Presence of Polypeptide Ligands

Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

Example 52: Identification Of Signal Transduction Proteins That Interact With An albumin fusion protein Of The Present Invention

Albumin fusion proteins of the invention may serve as research tools for the identification, characterization and purification of signal transduction pathway proteins or receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 53: IL-6 Bioassay

A variety of assays are known in the art for testing the proliferative effects of an albumin fusion protein of the invention. For example, one such asssay is the IL-6 Bioassay as described by Marz *et al.* (*Proc. Natl. Acad. Sci., U.S.A., 95*:3251-56 (1998), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls

containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μl, and 50 μl of fusion protein of the invention is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention) relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

Example 54: Support of Chicken Embryo Neuron Survival

To test whether sympathetic neuronal cell viability is supported by an albumin fusion protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A., 96*:11458-63 (1998), which is herein incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO_2 in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mosmann, T., *J. Immunol. Methods, 65*:55-63 (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

Example 55: Assay for Phosphatase Activity

The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [^{32}P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-labeled MyBP.

Example 56: Interaction of Serine/Threonine Phosphatases with other Proteins

Fusion protein of the invention having serine/threonine phosphatase activity (e.g., as determined in Example 55) are useful, for example, as research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein -complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 57: Assaying for Heparanase Activity

There a numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells (1×10^6 cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with ^{35}S-labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

Example 58: Immobilization of biomolecules

This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constucts (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation

upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO4 and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl2, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

Example 59: Assays for Metalloproteinase Activity

Metalloproteinases are peptide hydrolases which use metal ions, such as Zn^{2+}, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

Proteolysis of alpha-2-macroglobulin

To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases

Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND $HgCl_2$), peptide metalloproteinase inhibitors (TIMP-1 and TIMP–2), and commercial small molecule MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC_{50} = 1.0 μM against MMP-1 and MMP–8; IC_{50} = 30 μM against MMP-9; IC_{50} = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC_{50} = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50μg/ml) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M

NaCl, 10 mM $CaCl_2$, 25 μM $ZnCl_2$ and 0.05%Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1 μl of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

Synthetic Fluorogenic Peptide Substrates Cleavage Assay

The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques knonw in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor-α (TNF-α) converting enzyme (TACE). These substrastes are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μM. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μl 1x HEPES buffer (0.2 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μl of substrate solution (50 μM) at 25 °C for 15 minutes, and then adding 20 μl of a purified fusion protein of the invention into the assay cuvett. The final concentration of substrate is 1 μM. Initial hydrolysis rates are monitored for 30-min.

Example 60:Identification and Cloning of VH and VL domains

One method to identfy and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent (Life Technologies, Rockville. MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75%

ethanol. Follwing washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is the dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can determined using optical density measurements.

cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transciptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 3. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerse, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes are stored 4°C.

Table 3: Primer Sequences Used to Amplify VH and VL domains.

Primer name	SEQ ID NO	Primer Sequence (5'-3')
VH Primers		
Hu VH1-5'	36	CAGGTGCAGCTGGTGCAGTCTGG
Hu VH2-5'	37	CAGGTCAACTTAAGGGAGTCTGG
Hu VH3-5'	38	GAGGTGCAGCTGGTGGAGTCTGG
Hu VH4-5'	39	CAGGTGCAGCTGCAGGAGTCGGG
Hu VH5-5'	40	GAGGTGCAGCTGTTGCAGTCTGC
Hu VH6-5'	41	CAGGTACAGCTGCAGCAGTCAGG
Hu JH1,2-5'	42	TGAGGAGACGGTGACCAGGGTGCC
Hu JH3-5'	43	TGAAGAGACGGTGACCATTGTCCC
Hu JH4,5-5'	44	TGAGGAGACGGTGACCAGGGTTCC
Hu JH6-5'	45	TGAGGAGACGGTGACCGTGGTCCC
VL Primers		
Hu Vkappa1-5'	46	GACATCCAGATGACCCAGTCTCC
Hu Vkappa2a-5'	47	GATGTTGTGATGACTCAGTCTCC
Hu Vkappa2b-5'	48	GATATTGTGATGACTCAGTCTCC
Hu Vkappa3-5'	49	GAAATTGTGTTGACGCAGTCTCC
Hu Vkappa4-5'	50	GACATCGTGATGACCCAGTCTCC
Hu Vkappa5-5'	51	GAAACGACACTCACGCAGTCTCC
Hu Vkappa6-5'	52	GAAATTGTGCTGACTCAGTCTCC
Hu Vlambda1-5'	53	CAGTCTGTGTTGACGCAGCCGCC
Hu Vlambda2-5'	54	CAGTCTGCCCTGACTCAGCCTGC
Hu Vlambda3-5'	55	TCCTATGTGCTGACTCAGCCACC
Hu Vlambda3b-5'	56	TCTTCTGAGCTGACTCAGGACCC
Hu Vlambda4-5'	57	CACGTTATACTGACTCAACCGCC
Hu Vlambda5-5'	58	CAGGCTGTGCTCACTCAGCCGTC
Hu Vlambda6-5'	59	AATTTTATGCTGACTCAGCCCCA
Hu Jkappa1-3'	60	ACGTTTGATTTCCACCTTGGTCCC
Hu Jkappa2-3'	61	ACGTTTGATCTCCAGCTTGGTCCC
Hu Jkappa3-3'	62	ACGTTTGATATCCACTTTGGTCCC
Hu Jkappa4-3'	63	ACGTTTGATCTCCACCTTGGTCCC
Hu Jkappa5-3'	64	ACGTTTAATCTCCAGTCGTGTCCC
Hu Jlambda1-3'	65	CAGTCTGTGTTGACGCAGCCGCC
Hu Jlambda2-3'	66	CAGTCTGCCCTGACTCAGCCTGC
Hu Jlambda3--3'	67	TCCTATGTGCTGACTCAGCCACC
Hu Jlambda3b-3'	68	TCTTCTGAGCTGACTCAGGACCC
Hu Jlambda4-3'	69	CACGTTATACTGACTCAACCGCC
Hu Jlambda5-3'	70	CAGGCTGTGCTCACTCAGCCGTC
Hu Jlambda6-3'	71	AATTTTATGCTGACTCAGCCCCA

PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc., Carlsbad, CA). Individual cloned PCR products can be isolated after transfection of E. coli and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors containing the nucleotide sequences of a heavy (e.g., human IgG1 or human IgG4) or light chain (human kappa or human lambda) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional antibody molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domain to generate expression vectors that encode complete antibody molecules are well known within the art.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through Identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety. The specification and sequence listing of each of the following U.S. applications are herein incorporated by reference in their entirety: Application Nos. 09/091,873 filed June 25, 1998; 60/229,358 filed on April 12, 2000; 60/199,384 filed on April 25,2000; 60/256,931 filed on December 21, 2000, 09/809,269, filed March 16, 2001; 60/277,980, filed March 23, 2001; 09/236,557, filed January 26, 1999; 09/482,273, filed January 13, 2000; 60/234,925, filed November 1, 2000; 09/397,945, filed September 17, 1999; 09/296,622, filed April 23, 1999; 60/092,921, filed July 15, 1998; 09/305,736, filed May 5, 1999; 09/781,417, filed February 13, 2001; 60/152,317, filed September 3, 1999; 09/227,357, filed January 8, 1999; and 60/262,066,

filed January 18, 2001; and International Publication Nos. WO98/39446, filed September 11, 1998; WO 00/61625, filed October 19, 2000; WO/00/77022, filed December 21, 2000; and WO/00/76530, filed December 21, 2000.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	Unassigned

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

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The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

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The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: Unassigned

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The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

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The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	Unassigned

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
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Authorized officer	Authorized officer

Revised Form PCT/RO/134 (January 2001) Pctro134ep.sollist

ATCC Deposit No.: Unassigned

CANADA

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NORWAY

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AUSTRALIA

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FINLAND

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UNITED KINGDOM

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ATCC Deposit No.: Unassigned

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What is claimed:

1. An albumin fusion protein comprising a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:18.

2. An albumin fusion protein comprising a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity.

3. The albumin fusion protein of claim 2, wherein said albumin activity is the ability to prolong the shelf life of the Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused state.

4. The albumin fusion protein of claim 2, wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:18.

5. An albumin fusion protein comprising a fragment or variant of a Therapeutic protein:X, and albumin comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has a biological activity of the Therapeutic protein:X.

6. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin.

7. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin.

8. The albumin fusion protein of any one of claims 1-5, wherein the

Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C-terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin.

9. The albumin fusion protein of any one of claims 1-5, which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof.

10. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker.

11. The albumin fusion protein of any one of claims 1-8, wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant of albumin.

12. The albumin fusion protein of any one of claims 1-11, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the Therapeutic protein:X in an unfused state.

13. The albumin fusion protein of any one of claims 1-11, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

14. The albumin fusion protein of any one of claims 1-11, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

15. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

16. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising an amino acid sequence selected from the group consisting of:

(a) amino acids 54 to 61 of SEQ ID NO:18;
(b) amino acids 76 to 89 of SEQ ID NO:18;
(c) amino acids 92 to100 of SEQ ID NO:18;
(d) amino acids 170 to 176 of SEQ ID NO:18;
(e) amino acids 247 to 252 of SEQ ID NO:18;
(f) amino acids 266 to 277 of SEQ ID NO:18;
(g) amino acids 280 to 288 of SEQ ID NO:18;
(h) amino acids 362 to 368 of SEQ ID NO:18;
(i) amino acids 439 to 447 of SEQ ID NO:18;
(j) amino acids 462 to 475 of SEQ ID NO:18;
(k) amino acids 478 to 486 of SEQ ID NO:18; and
(l) amino acids 560 to 566 of SEQ ID NO:18.

17. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life of the Therapeutic

protein:X , or a fragment or variant thereof, in an unfused state.

18. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

19. The albumin fusion protein of claims 15 or 16 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

20. The albumin fusion protein of any one of claims 1-19, which is non-glycosylated.

21. The albumin fusion protein of any one of claims 1-19, which is expressed in yeast.

22. The albumin fusion protein of claim 21, wherein the yeast is glycosylation deficient.

23. The albumin fusion protein of claim 21 wherein the yeast is glycosylation and protease deficient.

24. The albumin fusion protein of any one of claims 1-19, which is expressed by a mammalian cell.

25. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

26. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein further comprises a secretion leader sequence.

27. A composition comprising the albumin fusion protein of any one of claims 1-26 and a pharmaceutically acceptable carrier.

28. A kit comprising the composition of claim 27.

29. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-26.

30. The method of claim 29, wherein the disease or disorder comprises indication:Y.

31. A method of treating a patient with a disease or disorder that is modulated by Therapeutic protein:X, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 1-26.

32. The method of claim 31, wherein the disease or disorder is indication:Y.

33. A method of extending the shelf life of Therapeutic protein:X comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin or a fragment or variant thereof, sufficient to extend the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

34. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-26.

35. A vector comprising the nucleic acid molecule of claim 34.

5

36. A host cell comprising the nucleic acid molecule of claim 35.

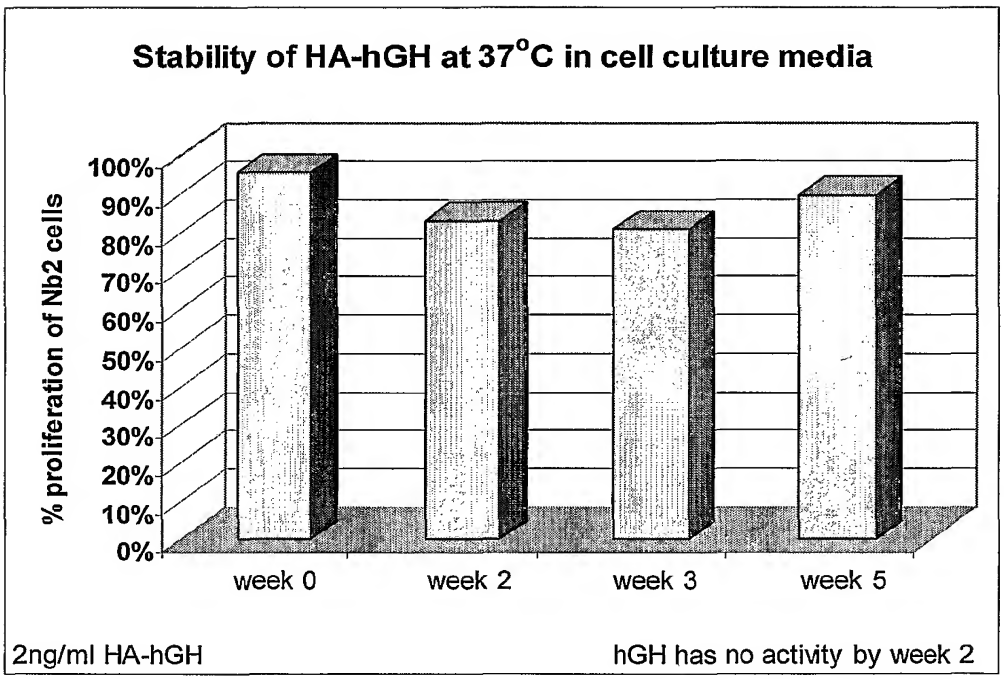

Stability of HA-hGH at 37°C in cell culture media
% proliferation of Nb2 cells
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
week 0
week 2
week 3
week 5
2ng/ml HA-hGH
hGH has no activity by week 2

Figure 1

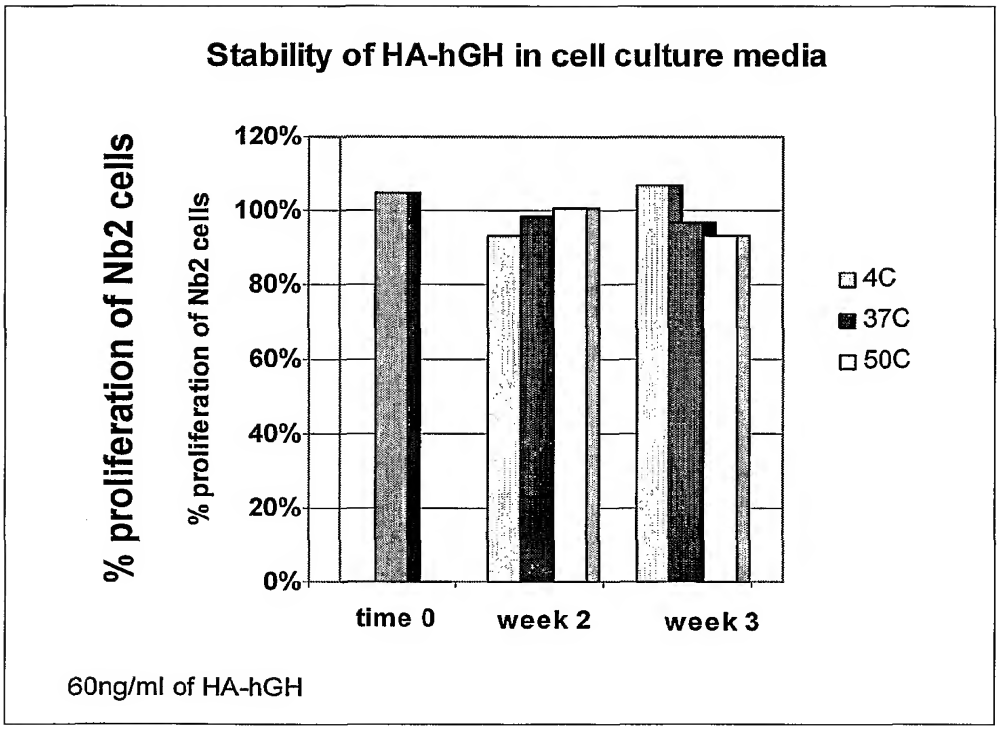

Stability of HA-hGH in cell culture media
% proliferation of Nb2 cells
% proliferation of Nb2 cells
120%
100%
80%
60%
40%
20%
0%
time 0
week 2
week 3
4C
37C
50C
60ng/ml of HA-hGH

Figure 2

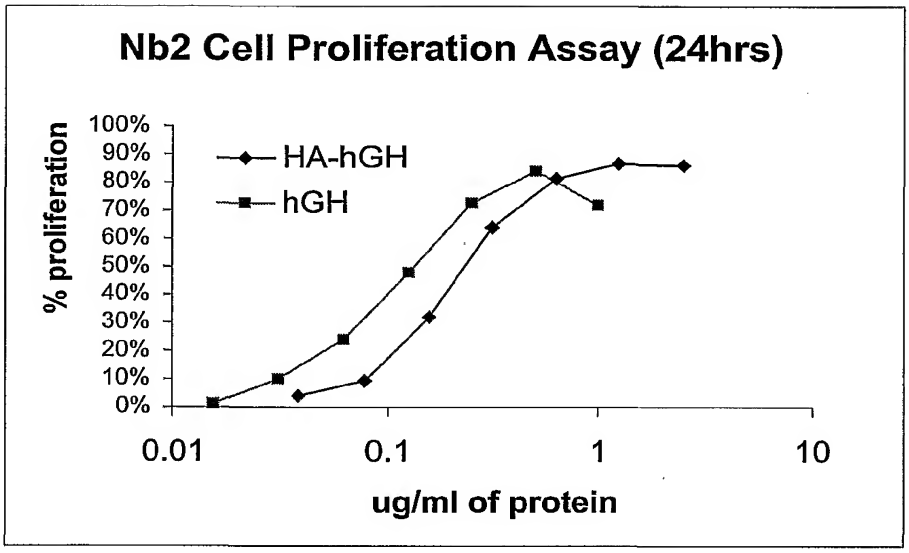


Figure 3A

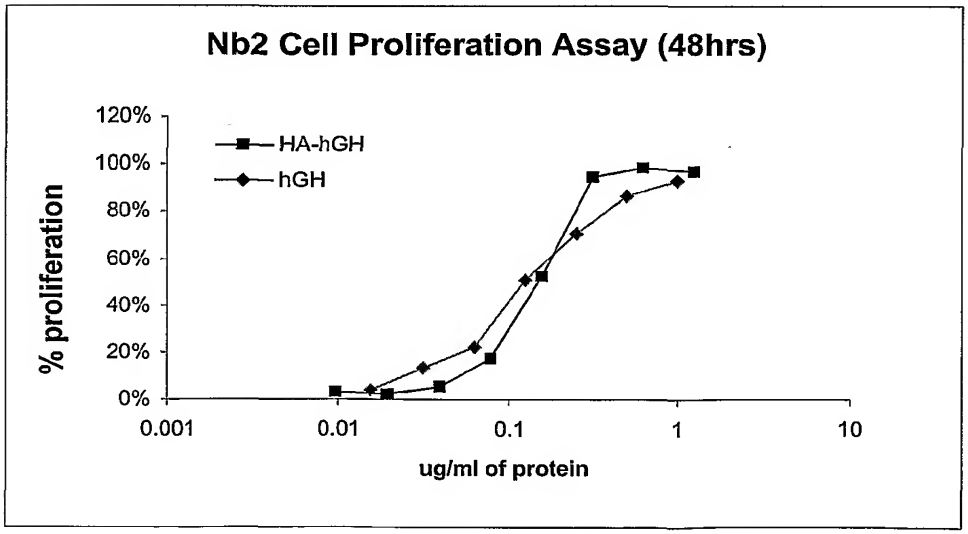
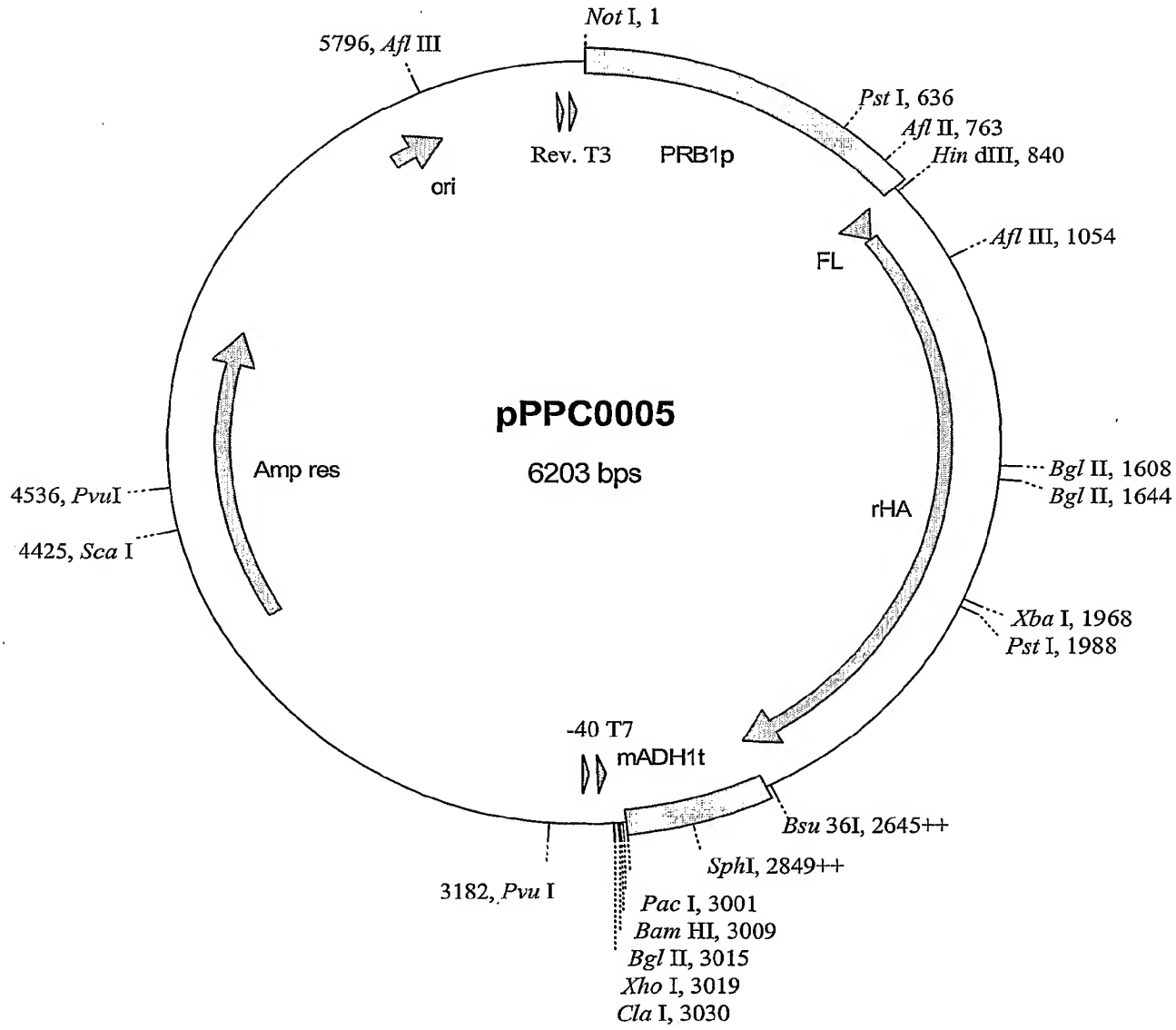


Figure 3B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Bsu 36I, 2645++
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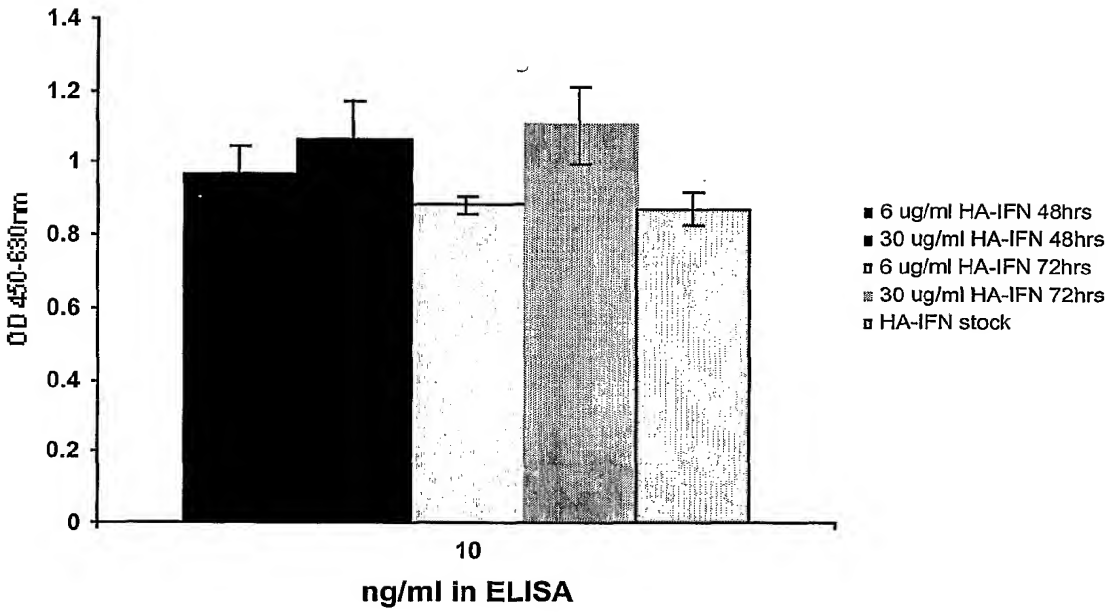
Cla I, 3030

Figure 4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1.2
1
0.8
0.6
0.4
0.2
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OD 450-630nm
10
ng/ml in ELISA
6 ug/ml HA-IFN 48hrs
30 ug/ml HA-IFN 48hrs
6 ug/ml HA-IFN 72hrs
30 ug/ml HA-IFN 72hrs
HA-IFN stock

Figure 5

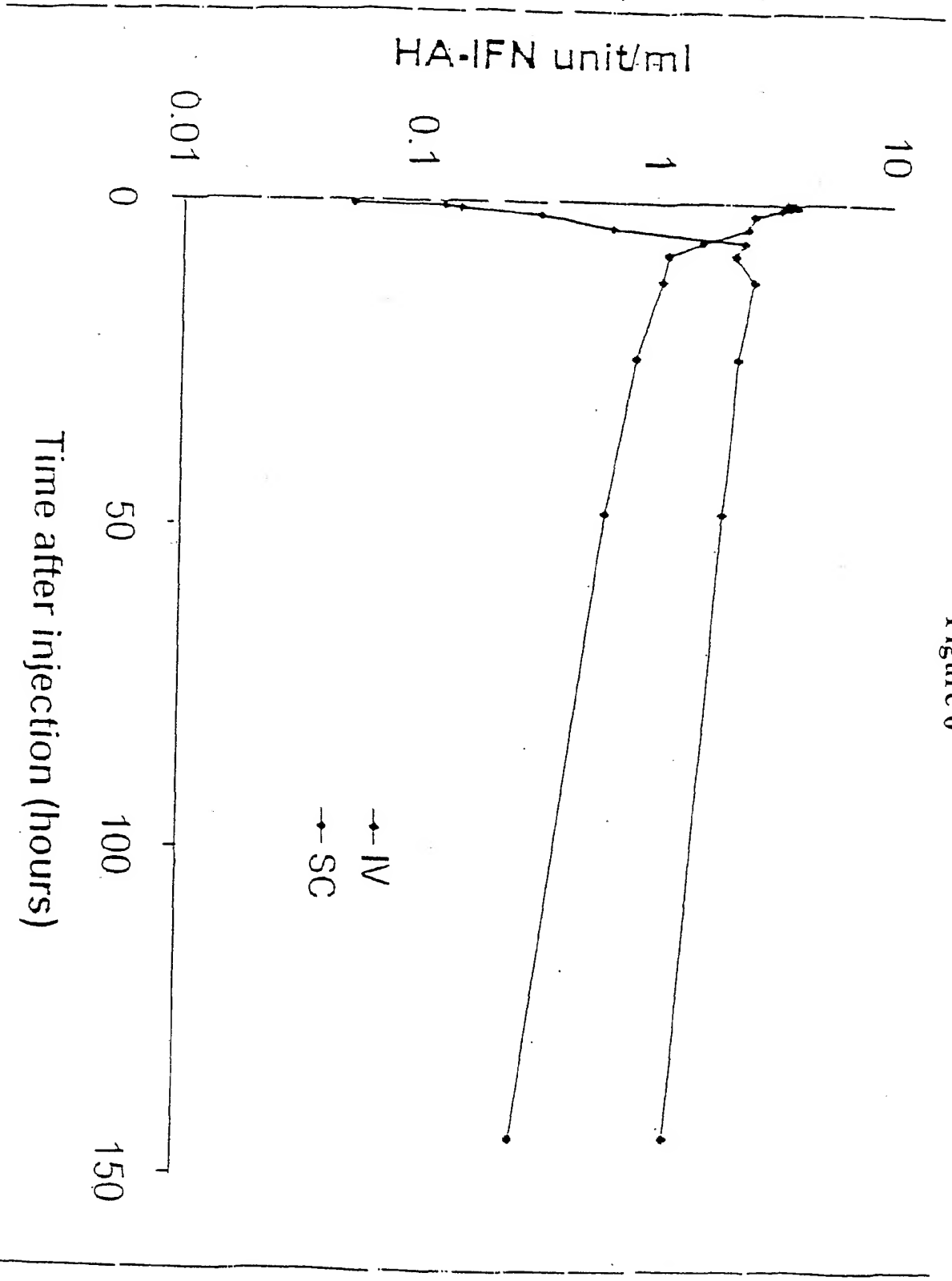

HA-IFN unit/ml
0.01
0.1
1
10
0
50
100
150
Time after injection (hours)
IV
SC

Figure 6

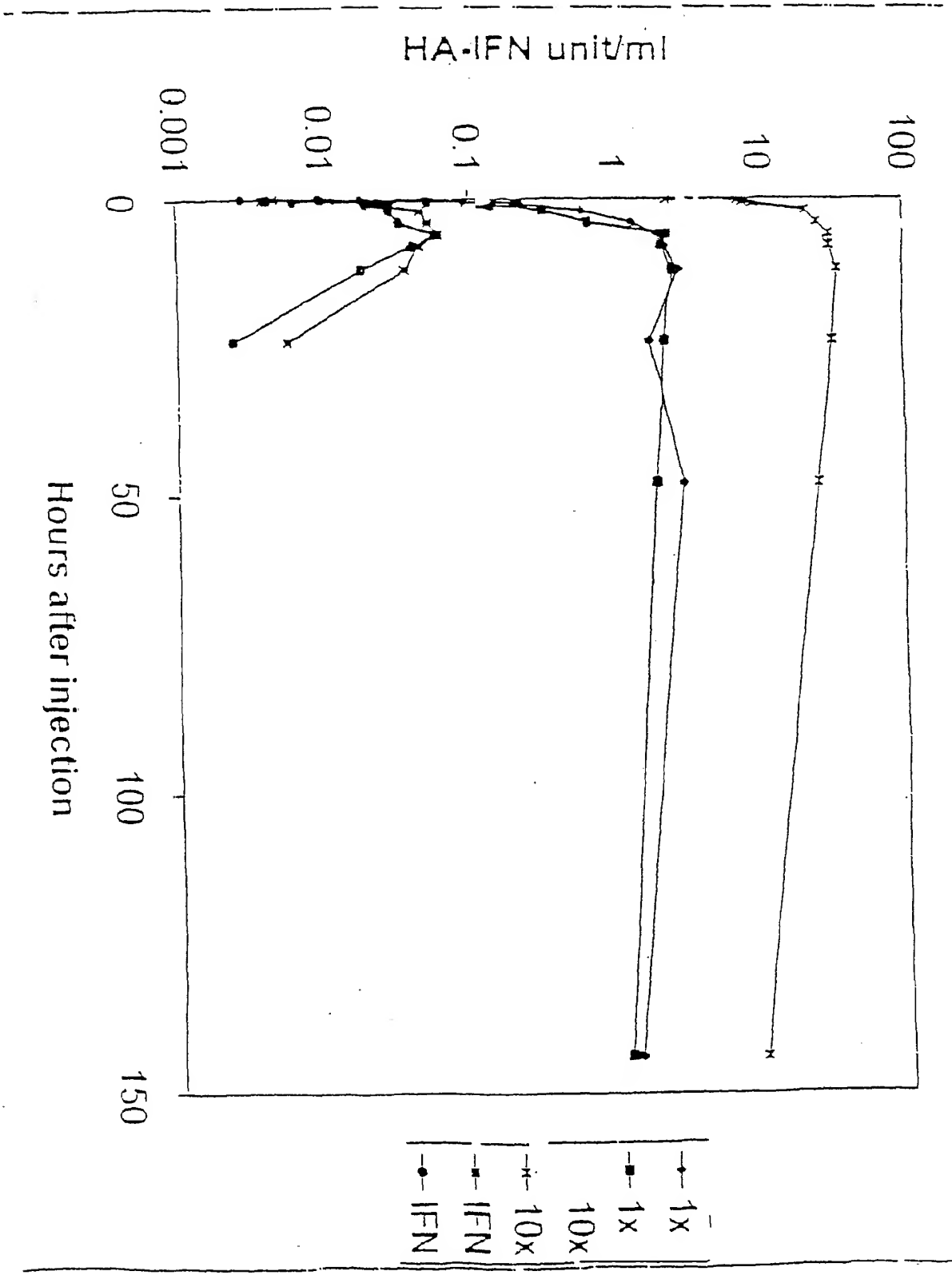
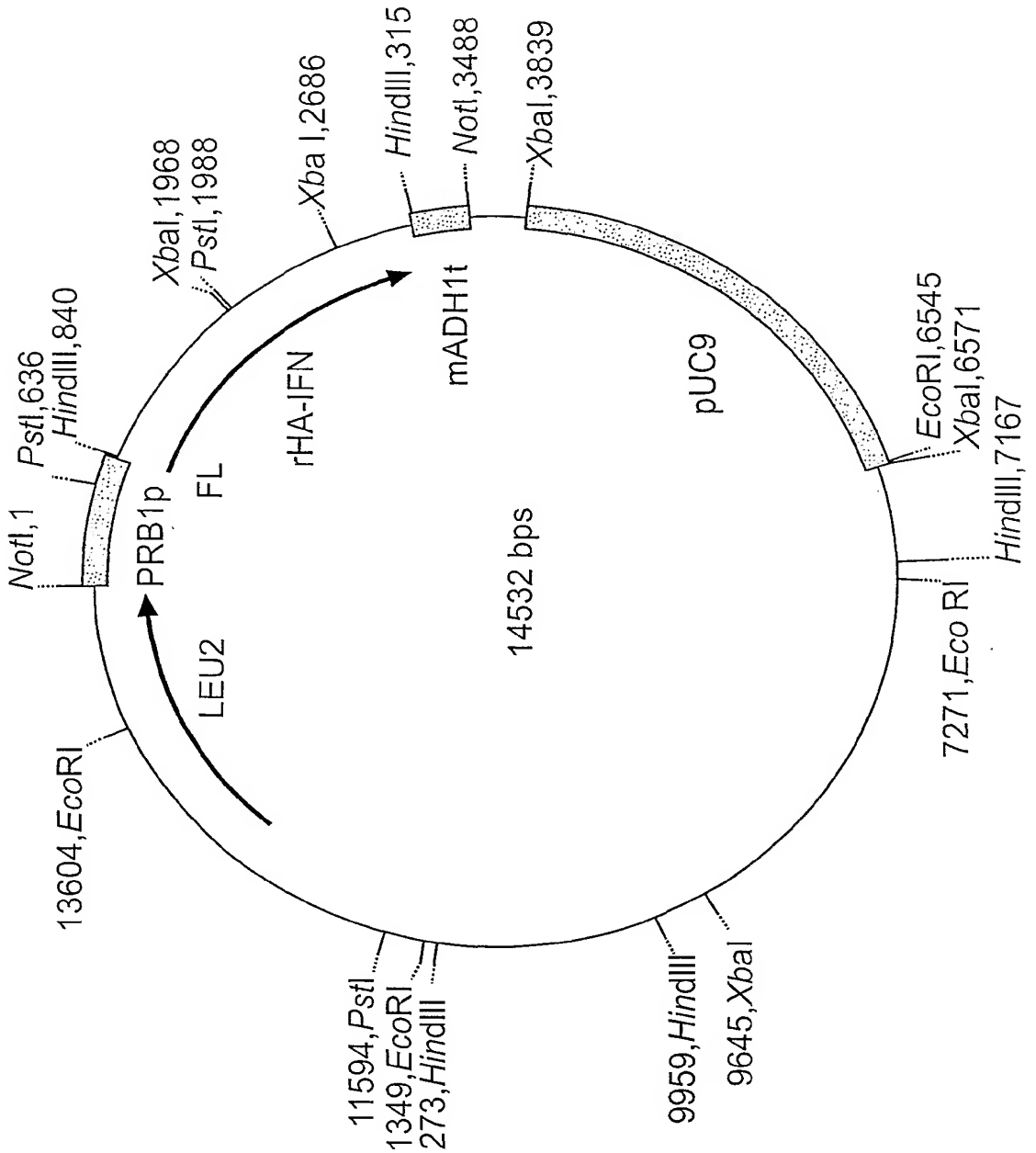

HA-IFN unit/ml
0.001
0.01
0.1
1
10
100
0
50
100
150
Hours after injection
IFN
IFN
10x
10x
1x
1x

Figure 7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HindIII,840
XbaI,1968
PstI,1988
Xba I,2686
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EcoRI,6545
XbaI,6571
HindIII,7167
7271,Eco RI
9645,XbaI
9959,HindIII
11594,PstI
1349,EcoRI
273,HindIII
13604,EcoRI
PRB1p
FL
rHA-IFN
mADH1t
pUC9
LEU2
14532 bps

FIG. 8

Localisation of 'Loops' based on the HA Crystal Structure which could be used for Mutation/Insertion

```
1     DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
           HHHHH HHH     HHH HHHHHHHHHH       HHHHH HHHHHHHHHH

                I                          II              III
51    KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
      HHHHH           HHHHH HHHHH              HHHH H     HHHH

101   CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
      HHHH                H HHHHHHHH    HHHHHHHHH HHHHH

                                IV
151   APELLFFAKR YKAAFTECCO AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHEHHHHHH HHHHHHHHHH

                                                          V
201   ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
      HHHHH   HH HHHHHHHHHH HH      HHH HHHHHHHHHH HHHHHH  HH

                          VI              VII
251   LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
      HHHHHHHHHH HHHHH           HHHHH     HHHHHHH H

301   DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
           HHHH      HHHHHH    HHHHHHH HHHHHH       HHHHHHHH

                   VIII
351   KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
      HHHHHHHHHH       HH    H   HHHHH HHHHHHHHHH HHHHHHH

                                                    IX
401   YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
      HHHHHHHHHH HHHH      H HHHHHHHHHH    HHH       HHHHHHH

                      X                     XI
451   DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
      HHHHHHHHHH HHHHH       HHHHHHHHH     HHHHHHHH

501   EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
                  HHH   HHH HHHHMMEHHH HHH          HHHHHHHH

                  XII
551   FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
      HHHHHHHH         HHHH HHHHHHHHHH HH
```

Loop		Loop	
I	Val54-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

```
                          IV
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHHHHHHHH HHHHHHHHHH

                          IV
151   APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHHHHHHHH HHHHHHHHHH
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$

↓

IV

```
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH       HHHHH HHHHHHHHHH HHHHHHHHHH
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

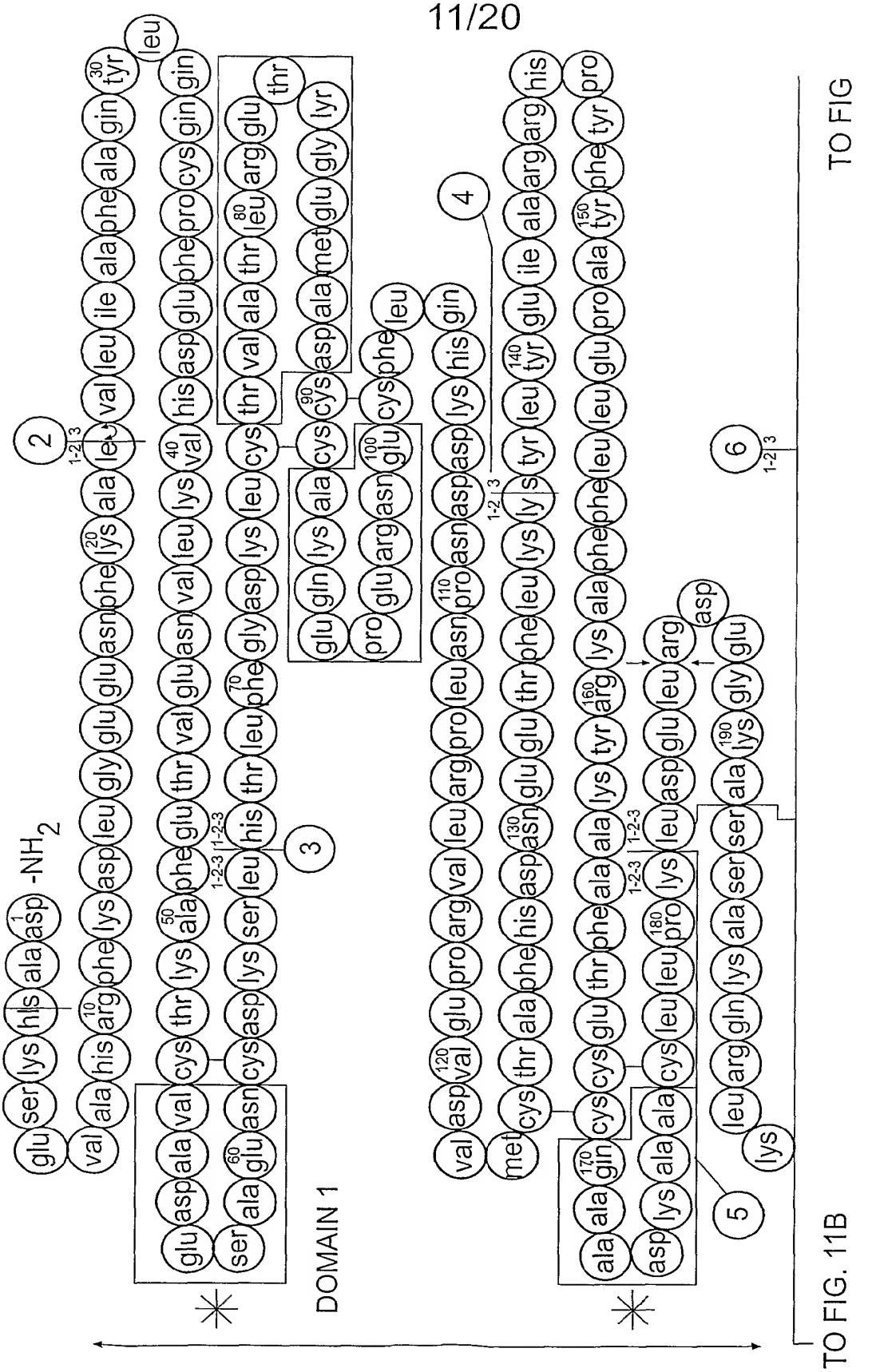

FIG. 11A

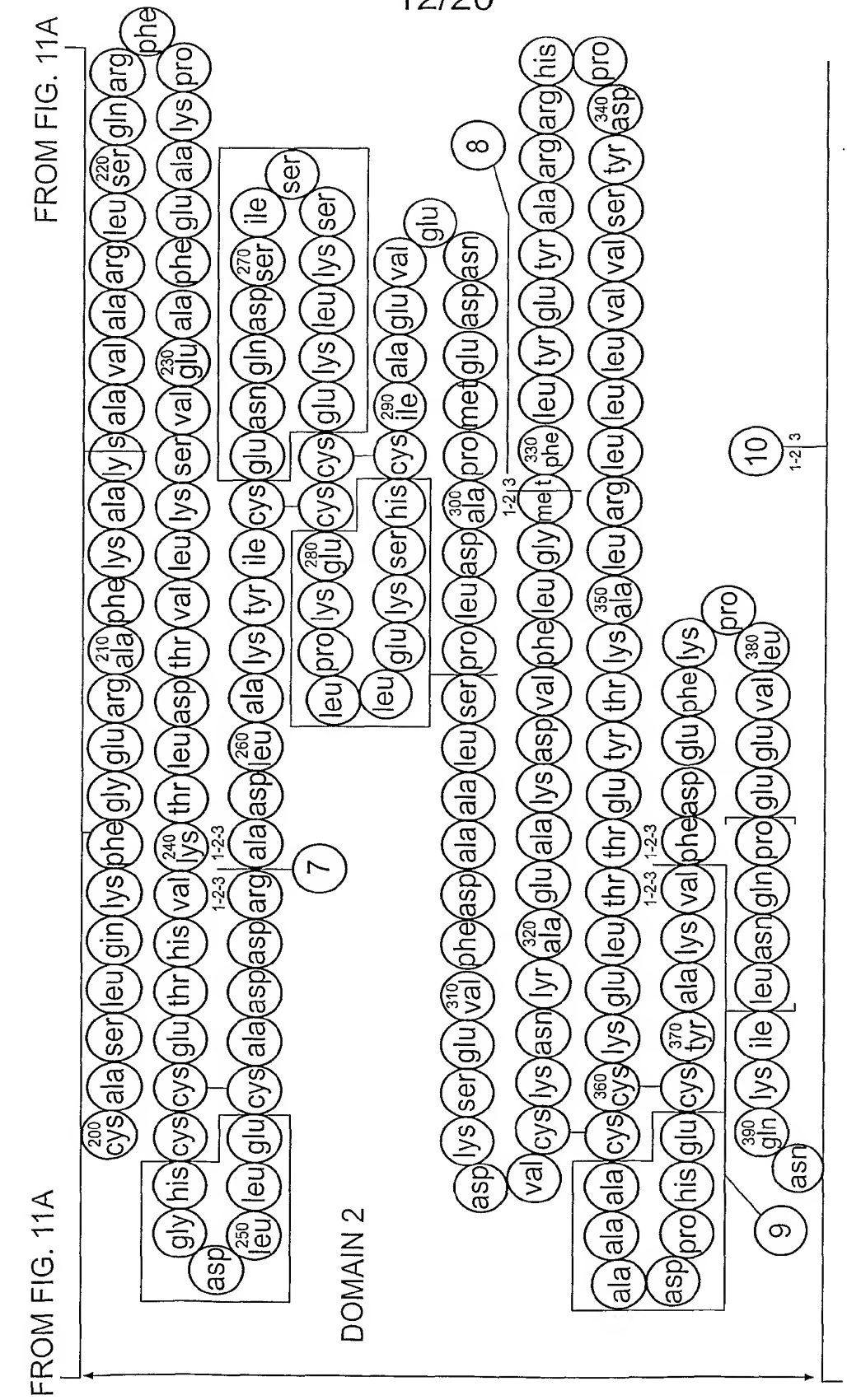

FIG. 11B

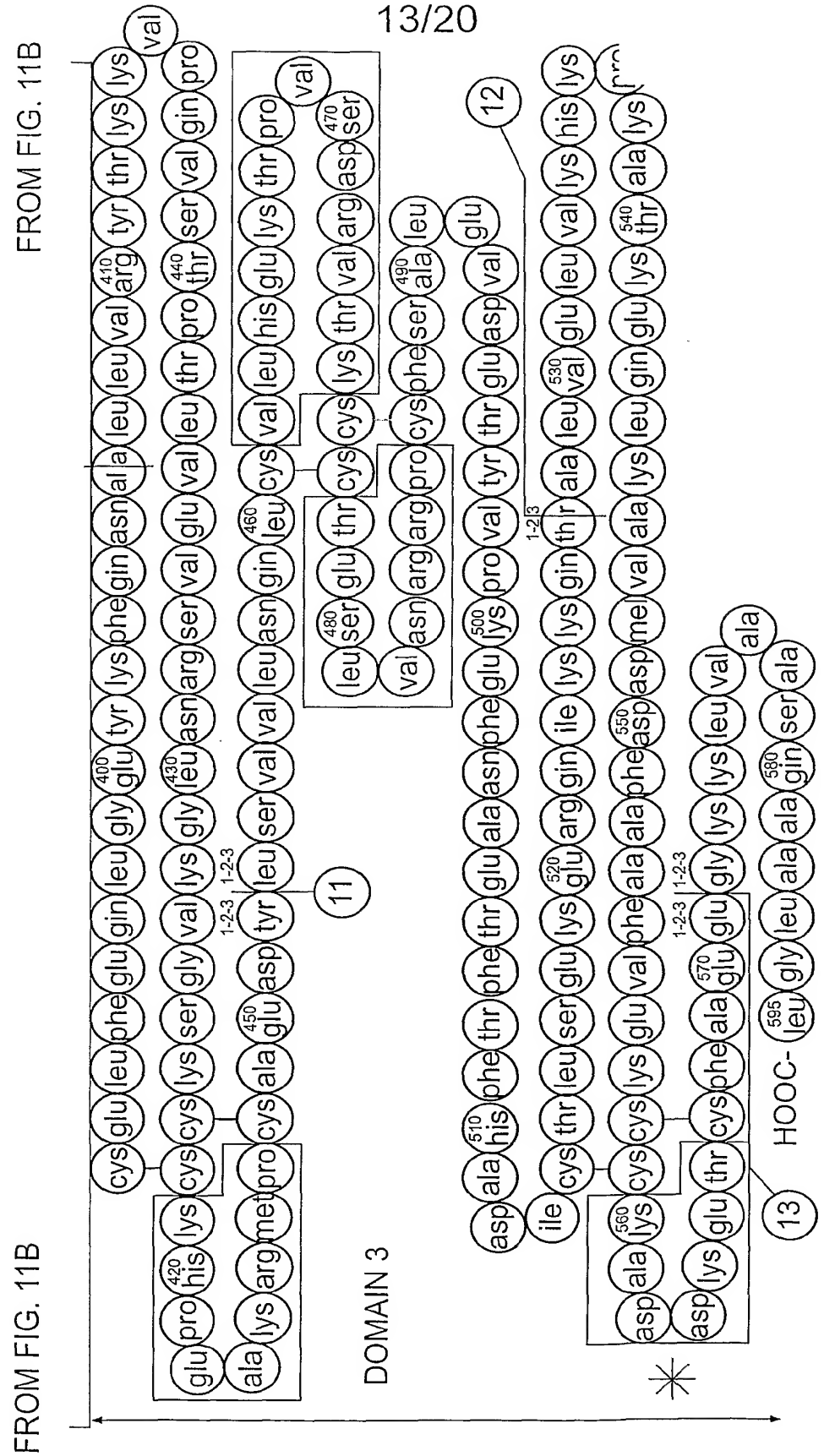

FIG. 11C

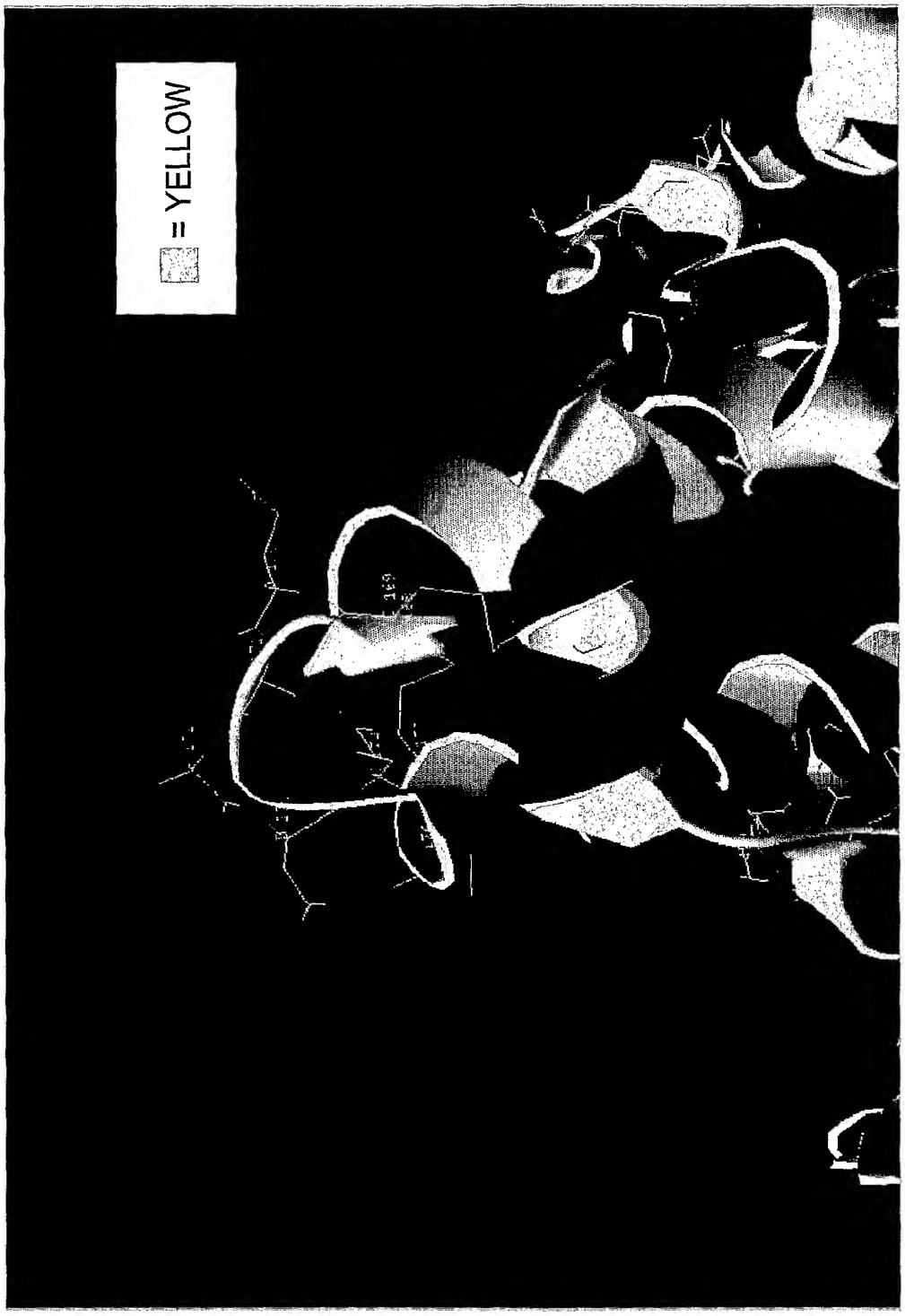

FIG. 12:
LOOP IV GLU170-A176

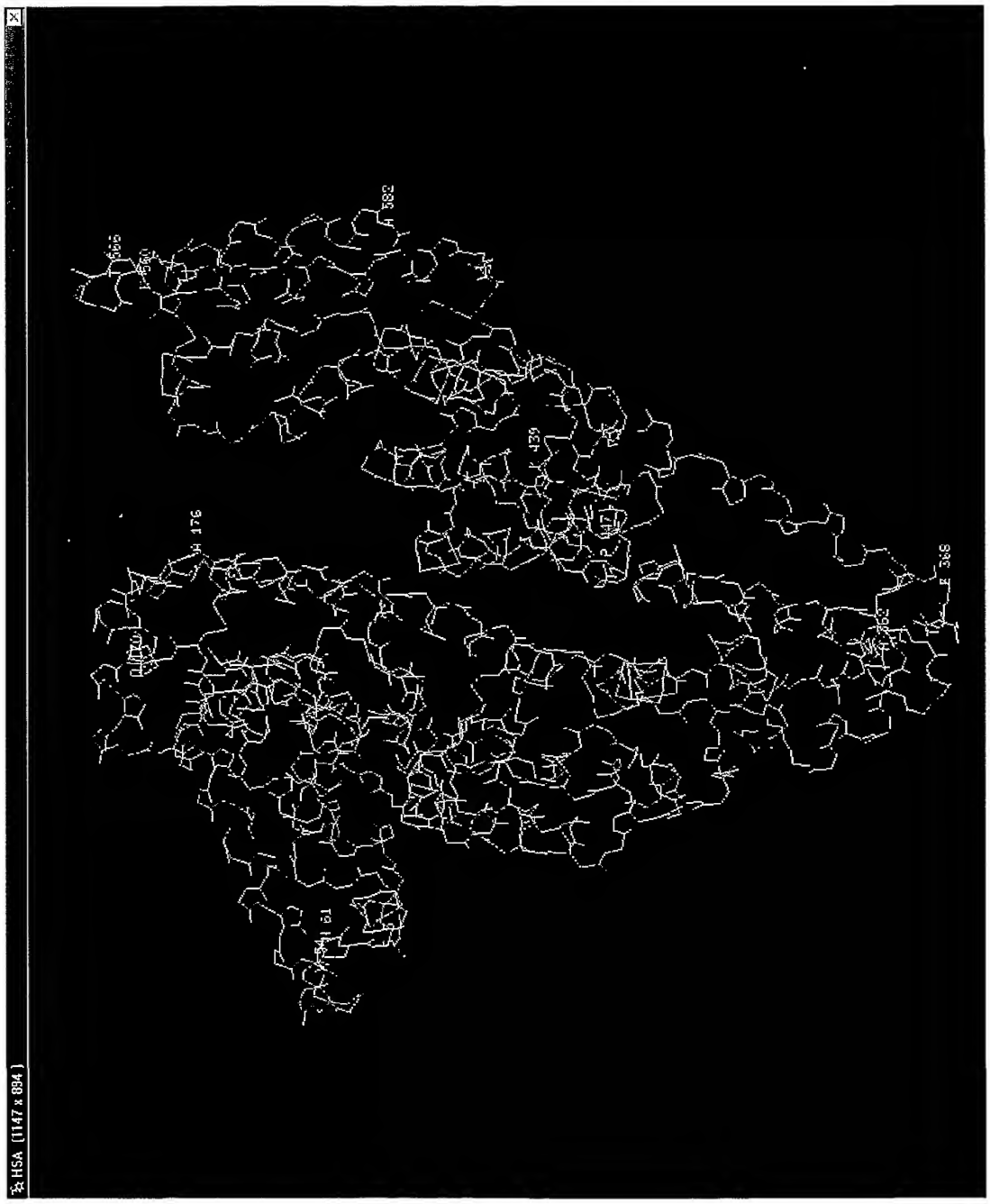

FIG. 13
TERTIARY STRUCTURE OF HA

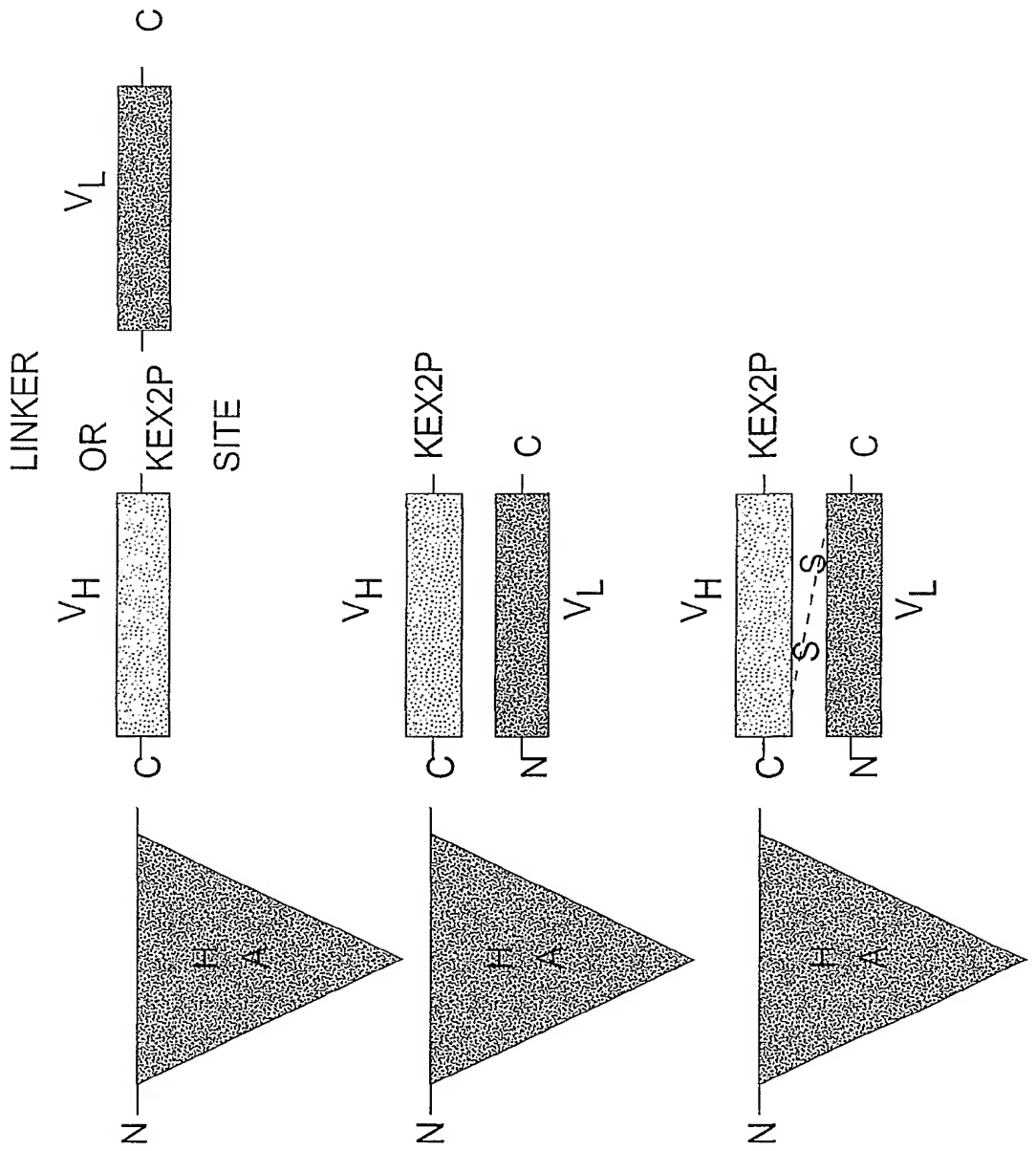

FIG. 14

```
   1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
   1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K  20

  61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
  21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V  40

 121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
  41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E  60

 181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
  61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L  80

 241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
  81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E  100

 301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
 101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V  120

 361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
 121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y  140

 421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
 141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R  160
```

Figure 15A

```
481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y   K   A   A   F   T   E   C   C   Q   A   A   D   K   A   A   C   L   L   P   180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K   L   D   E   L   R   D   E   G   K   A   S   S   A   K   Q   R   L   K   C   200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A   S   L   Q   K   F   G   E   R   A   F   K   A   W   A   V   A   R   L   S   220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q   R   F   P   K   A   E   F   A   E   V   S   K   L   V   T   D   L   T   K   240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V   H   T   E   C   C   H   G   D   L   L   E   C   A   D   D   R   A   D   L   260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A   K   Y   I   C   E   N   Q   D   S   I   S   S   K   L   K   E   C   C   E   280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K   P   L   L   E   K   S   H   C   I   A   E   V   E   N   D   E   M   P   A   300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D   L   P   S   L   A   A   D   F   V   E   S   K   D   V   C   K   N   Y   A   320
```

Figure 15B

```
 961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E   A   K   D   V   F   L   G   M   F   L   Y   E   Y   A   R   R   H   P   D   340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y   S   V   V   L   L   L   R   L   A   K   T   Y   E   T   T   L   E   K   C   360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C   A   A   A   D   P   H   E   C   Y   A   K   V   F   D   E   F   K   P   L   380

1141 GTG GAA GAG GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V   E   E   E   P   Q   N   L   I   K   Q   N   C   E   L   F   E   Q   L   G   E   400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y   K   F   Q   N   A   L   L   V   R   Y   T   K   K   V   P   Q   V   S   T   420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P   T   L   V   E   V   S   R   N   L   G   K   V   G   S   K   C   C   K   H   440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P   E   A   K   R   M   P   C   A   E   D   Y   L   S   V   V   L   N   Q   L   460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C   V   L   H   E   K   T   P   V   S   D   R   V   T   K   C   C   T   E   S   480
```

Figure 15C

```
1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L   V   N   R   R   P   C   F   S   A   L   E   V   D   E   T   Y   V   P   K   500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E   F   N   A   E   T   F   T   F   H   A   D   I   C   T   L   S   E   K   E   520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R   Q   I   K   K   Q   T   A   L   V   E   L   V   K   H   K   P   K   A   T   540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K   E   Q   L   K   A   V   M   D   D   F   A   A   F   V   E   K   C   C   K   560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A   D   D   K   E   T   C   F   A   E   E   G   K   K   L   V   A   A   S   Q   580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A   A   L   G   L   *                               585
```

Figure 15D

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<110> Human Genome Sciences, Inc.

<120> Albumin Fusion Proteins

<130> PF545PCT

<140> Unassigned
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<150> 60/229,358
<151> 2000-04-12

<150> 60/256,931
<151> 2000-12-21

<150> 60/199,384
<151> 2000-04-25

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<170> PatentIn Ver. 2.1

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with non-cohesive ends.

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<223> invertase leader sequence

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<223> first 5 amino acids of mature human serum albumin

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1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser
20

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<213> Artificial Sequence

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<210> 10
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<213> Artificial Sequence

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<400> 10
ctcttgtgtg catcgaagcc acag 24

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<213> Artificial Sequence

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<223> synthetic oligonucleotide used to join DNA fragments with non-cohesive ends.

<400> 11
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<210> 12
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<212> DNA
<213> Artificial Sequence

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aattgttggg aataaattct gaggctcttc c 31

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<400> 13
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<213> Artificial Sequence

<220>
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<400> 15

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ac 62

<210> 16
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
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<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

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gcc 63

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<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(1755)

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Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1 5 10 15

gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag 96
Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
20 25 30

cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa 144
Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35 40 45

ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50 55 60

tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt 240
Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65 70 75 80

cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85 90 95

gag aga aat gaa tgc ttc ttg caa cac aaa gat gac aac cca aac ctc 336
Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

ccc cga ttg gtg aga cca gag gtt gat gtg atg tgc act gct ttt cat 384
Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His

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        115                 120                 125

gac aat gaa gag aca ttt ttg aaa aaa tac tta tat gaa att gcc aga   432
Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

aga cat cct tac ttt tat gcc ccg gaa ctc ctt ttc ttt gct aaa agg   480
Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

tat aaa gct gct ttt aca gaa tgt tgc caa gct gct gat aaa gct gcc   528
Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

tgc ctg ttg cca aag ctc gat gaa ctt cgg gat gaa ggg aag gct tcg   576
Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

tct gcc aaa cag aga ctc aaa tgt gcc agt ctc caa aaa ttt gga gaa   624
Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

aga gct ttc aaa gca tgg gca gtg gct cgc ctg agc cag aga ttt ccc   672
Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

aaa gct gag ttt gca gaa gtt tcc aag tta gtg aca gat ctt acc aaa   720
Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

gtc cac acg gaa tgc tgc cat gga gat ctg ctt gaa tgt gct gat gac   768
Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

agg gcg gac ctt gcc aag tat atc tgt gaa aat cag gat tcg atc tcc   816
Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

agt aaa ctg aag gaa tgc tgt gaa aaa cct ctg ttg gaa aaa tcc cac   864
Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca   912
Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct   960
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga   1008
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

agg cat cct gat tac tct gtc gtg ctg ctg ctg aga ctt gcc aag aca   1056
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa   1104
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
```

```
        355                 360                 365

tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct   1152
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag   1200
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc   1248
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa   1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt   1344
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat   1392
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc   1440
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca   1488
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat   1536
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca   1584
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg   1632
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag   1680
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt   1728
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

gct gca agt caa gct gcc tta ggc tta taacatctac atttaaaagc atctcag 1782
Ala Ala Ser Gln Ala Ala Leu Gly Leu
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<210> 18

<211> 585
<212> PRT
<213> Homo Sapiens

<400> 18

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Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
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Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
            20                  25                  30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
        35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
    50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
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290 295 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305 310 315 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
325 330 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
340 345 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
355 360 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
370 375 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385 390 395 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
405 410 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
420 425 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
435 440 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
450 455 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465 470 475 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
485 490 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
500 505 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
515 520 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
530 535 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
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<210> 20
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site in pPPC0006

<400> 20
aatcgatgag caacctcact cttgtgtgca tctcttttct cgaggctcct ggaataag 58

<210> 21
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site in pPPC0006

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tacaaactta agagtccaat tagc 24

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site in pPPC0006

<400> 22
cacttctcta gagtggtttc atatgtctt 29

<210> 23
<211> 60
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<221> Misc_Structure
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<210> 24
<211> 60
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<220>
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sites in pPPC0007

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<210> 25
<211> 32
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<210> 26
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<223> reverse primer useful for generation of albumin fusion protein in which the albumin moiety is N-terminal

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ctttaaatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnnn nn 52

<210> 29
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> signal peptide of natural human serum albumin protein

<400> 29
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg
20

<210> 30
<211> 114
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<220>
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albumin fusion VECTOR

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<223> Hind III retsriction site

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<220>
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<223> cds natural signal sequence of human serum albumin

<220>
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<223> XhoI restriction site

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<223> cds first six amino acids of human serum albumin

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tcagggatcc aagcttccgc caccatgaag tgggtaacct ttatttccct tctttttctc 60

tttagctcgg cttactcgag gggtgtgttt cgtcgagatg cacacaagag tgag 114

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PC4:HSA albumin fusion VECTOR

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<223> Asp718 restriction site

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<400> 31
gcagcggtac cgaattcggc gcgccttata agcctaaggc agc 43

<210> 32
<211> 46
<212> DNA
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<220>
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<223> forward primer useful for inserting Therapeutic protein into pC4:HSA vector

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<210> 33
<211> 55
<212> DNA
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<210> 34
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1 5 10 15

Ala

<210> 35
<211> 22
<212> PRT
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<221> signal
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1 5 10 15

Trp Ala Pro Ala Arg Gly
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<210> 36
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<210> 37
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<212> DNA
<213> Artificial Sequence

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<400> 37
caggtcaact taagggagtc tgg 23

<210> 38
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<210> 39
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<212> DNA
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<210> 40
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<210> 41
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<210> 42
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<210> 43
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<210> 44
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<210> 45
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<213> Artificial Sequence

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amplifying human VH domains

<400> 45
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<210> 46
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amplifying human VL domains

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amplifying human VL domains

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amplifying human VL domains

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<210> 53
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<210> 54
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<213> Artificial Sequence

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<211> 23
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<213> Artificial Sequence

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<210> 72
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<221>turn
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<400> 72
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1 5 10 15

<210> 73
<211> 23
<212> PRT
<213> Homo sapiens

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Thr Cys Cys Ala Gly Gly Cys
20

<210> 74
<211> 429
<212> PRT
<213> Homo sapiens

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1 5 10 15

Ala Gly Ser Leu Gln Gly Lys Pro Leu Gln Ser Trp Gly Arg Gly Ser
20 25 30

Ala Gly Gly Asn Ala His Ser Pro Leu Gly Val Pro Gly Gly Gly Leu
35 40 45

Pro Glu His Thr Phe Asn Leu Lys Met Phe Leu Glu Asn Val Lys Val
50 55 60

Asp Phe Leu Arg Ser Leu Asn Leu Ser Gly Val Pro Ser Gln Asp Lys
65 70 75 80

Thr Arg Val Glu Pro Pro Gln Tyr Met Ile Asp Leu Tyr Asn Arg Tyr
85 90 95

Thr Ser Asp Lys Ser Thr Thr Pro Ala Ser Asn Ile Val Arg Ser Phe
100 105 110

Ser Met Glu Asp Ala Ile Ser Ile Thr Ala Thr Glu Asp Phe Pro Phe
115 120 125

Gln Lys His Ile Leu Leu Phe Asn Ile Ser Ile Pro Arg His Glu Gln
130 135 140

Ile Thr Arg Ala Glu Leu Arg Leu Tyr Val Ser Cys Gln Asn His Val
145 150 155 160

Asp Pro Ser His Asp Leu Lys Gly Ser Val Val Ile Tyr Asp Val Leu
165 170 175

Asp Gly Thr Asp Ala Trp Asp Ser Ala Thr Glu Thr Lys Thr Phe Leu
180 185 190

Val Ser Gln Asp Ile Gln Asp Glu Gly Trp Glu Thr Leu Glu Val Ser
195 200 205

Ser Ala Val Lys Arg Trp Val Arg Ser Asp Ser Thr Lys Ser Lys Asn

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    210                 215                 220

Lys Leu Glu Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu
225                 230                 235                 240

Asp Ile Ser Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val
                245                 250                 255

Phe Ser Asn Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu
            260                 265                 270

Arg Glu Met Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser
        275                 280                 285

Lys Asp Gly Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr
    290                 295                 300

Asp Gly His Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser
305                 310                 315                 320

Ala Gly Ala Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe
                325                 330                 335

Glu Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu
            340                 345                 350

Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val
        355                 360                 365

Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe
    370                 375                 380

Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro
385                 390                 395                 400

Ile Ser Val Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr
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His Tyr Glu Gly Met Ser Val Ala Glu Cys Gly Cys Arg
            420                 425

<210> 75
<211> 280
<212> PRT
<213> Homo sapiens

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Val Ile Thr Asp Glu Asn Trp Arg Glu Leu Leu Glu Gly Asp Trp Met
         35                  40                  45

Ile Glu Phe Tyr Ala Pro Trp Cys Pro Ala Cys Gln Asn Leu Gln Pro
     50                  55                  60

Glu Trp Glu Ser Phe Ala Glu Trp Gly Glu Asp Leu Glu Val Asn Ile
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65 70 75 80

Ala Lys Val Asp Val Thr Glu Gln Pro Gly Leu Ser Gly Arg Phe Ile
85 90 95

Ile Thr Ala Leu Pro Thr Ile Tyr His Cys Lys Asp Gly Glu Phe Arg
100 105 110

Arg Tyr Gln Gly Pro Arg Thr Lys Lys Asp Phe Ile Asn Phe Ile Ser
115 120 125

Asp Lys Glu Trp Lys Ser Ile Glu Pro Val Ser Ser Trp Phe Gly Pro
130 135 140

Gly Ser Val Leu Met Ser Ser Met Ser Ala Leu Phe Gln Leu Ser Met
145 150 155 160

Trp Ile Arg Thr Cys His Asn Tyr Phe Ile Glu Asp Leu Gly Leu Pro
165 170 175

Val Trp Gly Ser Tyr Thr Val Phe Ala Leu Ala Thr Leu Phe Ser Gly
180 185 190

Leu Leu Leu Gly Leu Cys Met Ile Phe Val Ala Asp Cys Leu Cys Pro
195 200 205

Ser Lys Arg Arg Arg Pro Gln Pro Tyr Pro Tyr Pro Ser Lys Lys Leu
210 215 220

Leu Ser Glu Ser Ala Gln Pro Leu Lys Lys Val Glu Glu Glu Gln Glu
225 230 235 240

Ala Asp Glu Glu Asp Val Ser Glu Glu Glu Ala Glu Ser Lys Glu Gly
245 250 255

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260 265 270

Pro Ser Leu Ala Thr Asp Lys Ser
275 280

<210> 76
<211> 112
<212> PRT
<213> *Homo sapiens*

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Phe Arg Leu Pro Arg Lys Trp Gly Trp Arg Thr Glu Ala Thr Ala Pro
20 25 30

His Ala Pro Val Pro Gln Ser Ile Cys Pro Arg Tyr Thr Ser Pro Cys
35 40 45

Ala Pro His Asp Cys Gly Ser Gln Thr Val Gln Gly Asn Ser Leu Ser
50 55 60

Leu Phe Tyr Thr Leu Ser His Lys Ala Pro Gln Leu Pro His Arg Val

65 70 75 80

Pro Ala Pro Leu Phe Cys Lys Tyr Val Lys Arg Lys Lys Cys Lys Arg
85 90 95

Trp Ser Leu Gly Trp Ser Ser Ser Leu Gln Leu Arg Leu Leu Thr Met
100 105 110

<210> 77
<211> 346
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<213> Homo sapiens

<400> 77
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Gly Trp Leu Leu Leu Leu Leu Leu Arg Gly Gly Ala Gln Ala Leu Glu
20 25 30

Cys Tyr Ser Cys Val Gln Lys Ala Asp Asp Gly Cys Ser Pro Asn Lys
35 40 45

Met Lys Thr Val Lys Cys Ala Pro Gly Val Asp Val Cys Thr Glu Ala
50 55 60

Val Gly Ala Val Glu Thr Ile His Gly Gln Phe Ser Leu Ala Val Arg
65 70 75 80

Gly Cys Gly Ser Gly Leu Pro Gly Lys Asn Asp Arg Gly Leu Asp Leu
85 90 95

His Gly Leu Leu Ala Phe Ile Gln Leu Gln Gln Cys Ala Gln Asp Arg
100 105 110

Cys Asn Ala Lys Leu Asn Leu Thr Ser Arg Ala Leu Asp Pro Ala Gly
115 120 125

Asn Glu Ser Ala Tyr Pro Pro Asn Gly Val Glu Cys Tyr Ser Cys Val
130 135 140

Gly Leu Ser Arg Glu Ala Cys Gln Gly Thr Ser Pro Pro Val Val Ser
145 150 155 160

Cys Tyr Asn Ala Ser Asp His Val Tyr Lys Gly Cys Phe Asp Gly Asn
165 170 175

Val Thr Leu Thr Ala Ala Asn Val Thr Val Ser Leu Pro Val Arg Gly
180 185 190

Cys Val Gln Asp Glu Phe Cys Thr Arg Asp Gly Val Thr Gly Pro Gly
195 200 205

Phe Thr Leu Ser Gly Ser Cys Cys Gln Gly Ser Arg Cys Asn Ser Asp
210 215 220

Leu Arg Asn Lys Thr Tyr Phe Ser Pro Arg Ile Pro Pro Leu Val Arg
225 230 235 240

Leu Pro Pro Pro Glu Pro Thr Thr Val Ala Ser Thr Thr Ser Val Thr

245 250 255

Thr Ser Thr Ser Ala Pro Val Arg Pro Thr Ser Thr Thr Lys Pro Met
260 265 270

Pro Ala Pro Thr Ser Gln Thr Pro Arg Gln Gly Val Glu His Glu Ala
275 280 285

Ser Arg Asp Glu Glu Pro Arg Leu Thr Gly Gly Ala Ala Gly His Gln
290 295 300

Asp Arg Ser Asn Ser Gly Gln Tyr Pro Ala Lys Gly Gly Pro Gln Gln
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Pro His Asn Lys Gly Cys Val Ala Pro Thr Ala Gly Leu Ala Ala Leu
325 330 335

Leu Leu Ala Val Ala Ala Gly Val Leu Leu
340 345

<210> 78
<211> 272
<212> PRT
<213> Homo sapiens

<400> 78
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Asp Glu Asp Ser Met Asp Ile Pro Leu Asp Leu Ser Ser Ser Ala Gly
20 25 30

Ser Gly Lys Arg Arg Arg Arg Gly Asn Leu Pro Lys Glu Ser Val Gln
35 40 45

Ile Leu Arg Asp Trp Leu Tyr Glu His Arg Tyr Asn Ala Tyr Pro Ser
50 55 60

Glu Gln Glu Lys Ala Leu Leu Ser Gln Gln Thr His Leu Ser Thr Leu
65 70 75 80

Gln Val Cys Asn Trp Phe Ile Asn Ala Arg Arg Arg Leu Leu Pro Asp
85 90 95

Met Leu Arg Lys Asp Gly Lys Asp Pro Asn Gln Phe Thr Ile Ser Arg
100 105 110

Arg Gly Ala Lys Ile Ser Glu Thr Ser Ser Val Glu Ser Val Met Gly
115 120 125

Ile Lys Asn Phe Met Pro Ala Leu Glu Glu Thr Pro Phe His Ser Cys
130 135 140

Thr Ala Gly Pro Asn Pro Thr Leu Gly Arg Pro Leu Ser Pro Lys Pro
145 150 155 160

Ser Ser Pro Gly Ser Val Leu Ala Arg Pro Ser Val Ile Cys His Thr
165 170 175

Thr Val Thr Ala Leu Lys Asp Val Pro Phe Ser Leu Cys Gln Ser Val

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Gly Val Gly Gln Asn Thr Asp Ile Gln Gln Ile Ala Ala Lys Asn Phe
        195                 200                 205

Thr Asp Thr Ser Leu Met Tyr Pro Glu Asp Thr Cys Lys Ser Gly Pro
    210                 215                 220

Ser Thr Asn Thr Gln Ser Gly Leu Phe Asn Thr Pro Pro Pro Thr Pro
225                 230                 235                 240

Pro Asp Leu Asn Gln Asp Phe Ser Gly Phe Gln Leu Leu Val Asp Val
                245                 250                 255

Ala Leu Lys Arg Ala Ala Glu Met Glu Leu Gln Ala Lys Leu Thr Ala
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<210> 79
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<213> Homo sapiens

<400> 79

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Asn Ala Ile Gln Ala Arg Ser Ser Ser Tyr Ser Gly Glu Tyr Gly Gly
             20                  25                  30

Gly Gly Gly Lys Arg Phe Ser His Ser Gly Asn Gln Leu Asp Gly Pro
         35                  40                  45

Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu
     50                  55                  60

Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn
 65                  70                  75                  80

Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln
                85                  90                  95

Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr
            100                 105                 110

Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe
        115                 120                 125

Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly
    130                 135                 140

Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr
145                 150                 155                 160

Pro Thr Ser Cys Ser Arg Cys
                165
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	PTA-3276

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only		For International Bureau use only	
☐ This sheet was received with the international application		☐ This sheet was received by the International Bureau on	
Authorized officer		Authorized officer	

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ATCC Deposit No.: PTA-3276

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-3276

DENMARK

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later that at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☒

Name of depositary institution: American Type Culture Collection

Address of depositary institution *(including postal code and country)*
10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	PTA-3277

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

Europe
In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC). Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the international Bureau later *(specify the general nature of the indications e.g. "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on
Authorized officer	Authorized officer

Revised Form PCT/RO/134 (January 2001) Pcro134ep.sollist

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11941

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 37/02; C12N 15/00
US CL : 530/350; 435/7.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
EST, GENEMBL, AGENESEQ

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE, BIOSIS, USPAT, JAPIO, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/23857 A1 (DELTA BIOTECHNOLOGY LIMITED) 08 September 1995 (08.09.95), see entire document.	1-9, 15-19
X	EP 0 322 094 A1 (DELTA BIOTECHNOLOGY LIMITED) 28 June 1989 (28.06.89), see entire document.	1-9, 15-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 JULY 2001	Date of mailing of the international search report 14 AUG 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer HOPE ROBINSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11991

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10-14, 20-32 and 34-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 15-19 (FGF-7)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/11991

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Groups 1-58, claim(s) 1-9, 15-19, all in part, drawn to a therapeutic protein X, wherein X correlates to those listed in the Table on page 11 of the description. If any of Groups 1-58 are elected the claims will only be examined in-so-far-as it pertains to the elected protein X. For example,
If Group 1 is elected, this correlates to Therapeutic Protein X:FGF-7.
Group 2, all partially as in Group 1, concerning Therapeutic Protein X: Kertainocyte growth factor 2.
Group 3, all partially as in Group 1, concerning Therapeutic Protein X: C-C Chemokine Receptor 5.
Group 4, all partially as in Group 1, concerning Therapeutic Protein X: Cathepsin K.
Group 5, all partially as in Group 1, concerning Therapeutic Protein X: MPIF-1 (Myeloid Progenitor Inhibitory Factor).
Group 6, all partially as in Group 1, concerning Therapeutic Protein X: VEGF.
Group 7, all partially as in Group 1, concerning Therapeutic Protein X: VEGF-2.
Group 8, all partially as in Group 1, concerning Therapeutic Protein X: BLyS.
Group 9, all partially as in Group 1, concerning Therapeutic Protein X: KD1.
Group 10, all partially as in Group 1, concerning Therapeutic Protein X: TMP-1.
Group 11, all partially as in Group 1, concerning Therapeutic Protein X: TMP-2.
Group 12, all partially as in Group 1, concerning Therapeutic Protein X: TMP-3.
Group 13, all partially as in Group 1, concerning Therapeutic Protein X: TMP-4.
Group 14, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor Protein.
Group 15, all partially as in Group 1, concerning Therapeutic Protein X:CTGF-2.
Group 16, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor 4.
Group 17, all partially as in Group 1, concerning Therapeutic Protein X: Human T-cell Lymphoma-lipoprotein associated phospholipase-A2.
Group 18, all partially as in Group 1, concerning Therapeutic Protein X: VEGI.
Group 19, all partially as in Group 1, concerning Therapeutic Protein X: AIM-1.
Group 20, all partially as in Group 1, concerning Therapeutic Protein X: TNF-delta.
Group 21, all partially as in Group 1, concerning Therapeutic Protein X: TNF-epsilon.
Group 22, all partially as in Group 1, concerning Therapeutic Protein X: AIM-2.
Group 23, all partially as in Group 1, concerning Therapeutic Protein X: Endokine.
Group 24, all partially as in Group 1, concerning Therapeutic Protein X: TR1.
Group 25, all partially as in Group 1, concerning Therapeutic Protein X:TR2.
Group 26, all partially as in Group 1, concerning Therapeutic Protein X: DR3.
Group 27, all partially as in Group 1, concerning Therapeutic Protein X: TR4.
Group 28, all partially as in Group 1, concerning Therapeutic Protein X: 4-1BBsv receptor.
Group 29, all partially as in Group 1, concerning Therapeutic Protein X: OPGL.
Group 30, all partially as in Group 1, concerning Therapeutic Protein X: FasL.
Group 31, all partially as in Group 1, concerning Therapeutic Protein X: Fas.
Group 32, all partially as in Group 1, concerning Therapeutic Protein X: TR5.
Group 33, all partially as in Group 1, concerning Therapeutic Protein X: TR6.
Group 34, all partially as in Group 1, concerning Therapeutic Protein X: DR5.
Group 35, all partially as in Group 1, concerning Therapeutic Protein X: TR8.
Group 36, all partially as in Group 1, concerning Therapeutic Protein X: TR9.
Group 37, all partially as in Group 1, concerning Therapeutic Protein X: TR10.
Group 38, all partially as in Group 1, concerning Therapeutic Protein X: TR11.
Group 39, all partially as in Group 1, concerning Therapeutic Protein X: TR12.
Group 40, all partially as in Group 1, concerning Therapeutic Protein X: TR13.
Group 41, all partially as in Group 1, concerning Therapeutic Protein X: TR14.
Group 42, all partially as in Group 1, concerning Therapeutic Protein X: TR16.
Group 43, all partially as in Group 1, concerning Therapeutic Protein X: HLDOU18.
Group 44, all partially as in Group 1, concerning Therapeutic Protein X: HSDSB09.
Group 45, all partially as in Group 1, concerning Therapeutic Protein X: HDPBQ71.
Group 46, all partially as in Group 1, concerning Therapeutic Protein X: HAGDG59.
Group 47, all partially as in Group 1, concerning Therapeutic Protein X: HCHNF25.
Group 48, all partially as in Group 1, concerning Therapeutic Protein X: HKACD58.
Group 49, all partially as in Group 1, concerning Therapeutic Protein X: HWACB86.
Group 50, all partially as in Group 1, concerning Therapeutic Protein X: HFTCF50.
Group 51, all partially as in Group 1, concerning Therapeutic Protein X: HRDFD27.
Group 52, all partially as in Group 1, concerning Therapeutic Protein X: HCEGG08.
Group 53, all partially as in Group 1, concerning Therapeutic Protein X: HKACI79.

Group 54, all partially as in Group 1, concerning Therapeutic Protein X: HWHGZ51.
Group 55, all partially as in Group 1, concerning Therapeutic Protein X: HDTAI21.
Group 56, all partially as in Group 1, concerning Therapeutic Protein X: HCNCA73.
Group 57, all partially as in Group 1, concerning Therapeutic Protein X: HNHFE71.
Group 58, all partially as in Group 1, concerning Therapeutic Protein X: HLWCF05.

Group 59-117, claim(s) 33, drawn to a method of extending shelf life of Therapeutic Protein X, wherein Therapeutic Protein X can be any of the proteins listed in the Table on page 11 of the description. If any of Groups 59-117 are elected the claim will only be examined in-so-far-as it pertains to the elected Protein X.

The inventions listed as Groups 1-118 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: because the technical feature linking groups 1-118 is not special because Inventions 1-58 do not avoid the prior art as Delta Biotechnology Limited, (EP 322094, June 28, 1989) teaches the claimed sequence. Thus, the invention does not relate to a single inventive concept and is not a contribution over the prior art.